Determination of protein concentration using a micro- Kjeldahl procedure[1]

Reagents

(1) Digestion mixture: **CAUTION!** These reagents are highly corrosive and toxic.

0.4 ml selenium oxychloride (Aldrich # 336157 http://www.sigmaaldrich.com/)

8 gm potassium sulfate (Sigma-Aldrich P0772 http://www.sigmaaldrich.com/)

50 ml concentrated sulfuric acid (Fisher A468-250 (https://www1.fishersci.com/)

and water to 250 ml total

(2) Standard: Solutions of ammonium chloride (Acros AC42328-0010

https://www1.fishersci.com) containing between 10-80 micrograms of nitrogen in 2 ml glass distilled or deionized water.

(3) hydrogen peroxide (30%) (Sigma Aldrich H1009).

(4) Color Reagent:

Nessler's Reagent (Fisher SN16I-500 250 https://www1.fishersci.com/)

PROCEDURE

1 Digest protein samples containing 20-80 micrograms of nitrogen with 0.5 ml of digestion mixture in 15 ml heavy walled Pyrex graduated tubes (Aldrich CLS808015) at 100-200 ^{o}C in a sand bath (tray filled with sand on a heavy duty hot plate) until the solvent evaporates.

2 Raise the temperature to 300-315 ^{o}C for 1-5 hours until the solution becomes colorless (the material will first turn, brown, then green, then clear).

3 Cool to room temperature and add approximately 0.2 ml of hydrogen peroxide (30%) (Sigma Aldrich H1009).

4 Heat the samples for an additional hour at 300 ^{o}C.

5 Cool to room temperature and bulk to 10 ml with glass distilled water.

6 Take two 1-ml aliquots for analysis. Add 1 ml of water and 1 ml of alkaline Nessler's Reagent (Fisher).

7 Let the color intensity develop for 10 minutes and measure the intensity at 420 nm.

8 The concentration of the protein is determined by calculating the percentage nitrogen from its amino acid composition.

REFERENCE

1. Lang, C. A.(1958) Simple microdetermination of Kjeldahl nitrogen in biological material. Anal. Chem. *30*, 1692-1694.